BIOACTIVITY GUIDED ANTIMICROBIAL POTENTIALS OF SELECTED ARID ZONE PLANTS OF RAJASTHAN

Research Protocol

For Registration in Ph.D. programme

to

JECRC UNIVERSITY, JAIPUR

Submitted by

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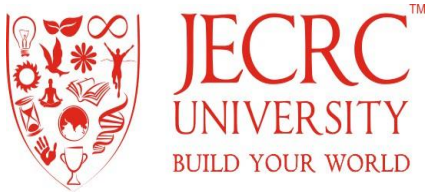

SCHOOL OF SCIENCE

JECRC UNIVERSITY

March 2023

JECRC UNIVERSITY, JAIPUR

Dated:

CERTIFICATE

This is to certify that this Research Protocol entitled **"BIOACTIVITY GUIDED ANTIMICROBIAL POTENTIALS OF SELECTED ARID ZONE PLANTS OF RAJASTHAN"** by **TRIPTI GAUTAM,** submitted in fulfilment of the requirement for registration in Ph.D. Program in Department of Biotechnology under School of Science of JECRC University, Jaipur, during the academic year 2023, is a bonafied record work carried out under our guidance and supervision.

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INTRODUCTION

Plants are one of the main multicellular organisms which play an important role in the preservation on earth's life. They prepare their food by using the process of photosynthesis. The earth has about 3, 20,000 species of plants. These plants have characteristic to produce various bioactive molecules and converting them into a great source of medicines. There are so many drugs on the planet which are produced from plants. (Sukanya et al., 2009). In developing nations, plants work as a rich source of drugs (Vulto and Smet, 1988). It can be stated that plants are the major resource for herbal and medical industry. These medicines have been prepared by conducting research. In addition to this, plants play a significant role in preparing food for animals and human beings.

Basically, there are five categories of plants such as shrubs, herbs, creepers, trees and climbers. Each and every part of a plant like root, stem, leaves have different roles in medicines. Some of the plants have characteristics which assist to treat and heal wounds and disorders and they can also be used as nutritional elements. These plants are known as medicinal plants. Medicinal plans are used all over the world for treating many diseases like skin disorders, asthma, gastrointestinal issues, urinary and respiratory issues and cardiovascular disorders (Van and Wink ,2004).

Some of the medicinal plants with their botanical names and uses are given below:

English Name	**Scientific/Botanical Name**	**Uses**
Coriander	*Coriandrum sativum*	Indigestion and Flatulence
Kalmegh	*Andrographi spaniculata*	Acne and Diarrhoea
Garlic	*Allium sativum*	Wounds and Dysentery
Basil	*Ocimum basilicum*	Indigestion, Heart & Respiratory Diseases
Cardamom	*Elettaria cardamomum*	Vomiting and Nausea
Aloes	*Aloe vera*	Ulcers and Acne
Long Pepper	*Piper longum*	Asthma, Cough indigestion Indigestion
Indian Olibanum	*Boswellia serrate*	Joint Pains and Diabetes
Cinnamon Leaf	*Cinnamomum tamala*	Diabetes, Digestion and Cold

A wide range of biologically active compounds is synthesized from these medicinal plants (Bajguz, 2007). It is very important to identify the bio active compounds in order to examine the antimicrobial properties of plants related to dermatology disorders. The creation of bio active compounds differs on the basis of soil types, species of plant and their relation with microorganisms (Zhao et al., 2011). A report of World Health Organization (WHO) presents that medical plants are the great source to formulate different types of drugs (Nascimento et al., 2000). The medicinal significance of plants presents in some chemical elements which create a physiological activity on human body. These substances are known as phytochemicals. They are used in homeopathic and herbal medicines to cure various diseases (Chitravadivu et al., 2009). These substances have non-nutritive, disease preventive and protective characteristics (Ahmed and Urooj, 2009).

So, there occurs a requirement to screen medicinal plants for biologically active compounds as the base for advanced studies.

Some biologically active compounds with their origin are as follows: (Comforti et al., 2009)

S.no.	Bio- active compounds	Origin	Action mechanisms
1	β- carotene	Vegetables and fruits	Recovery of heart disease by the use of antioxidants
2	Catechin	Green tea	Antioxidant
3	Flavonoids	Vegetables (mint)	Anti-proliferative activity of MCF7

LITERATURE REVIEW

Plants are one of the most significant living organisms on earth. They play an important role to both human beings and animals as they make oxygen for their survival. The major parts of a plant are root, leaves, stem, flowers and fruits. There are so many plants on earth which are used for medicinal purposes.

Tephrosia purpurea is one of the best example of medicinal plants that is known as ‘sharpunkha’. It is from the family fabaceae and subfamily papilionaceae. This genus includes around 400 species. This plant is herbaceous, highly branched and perennial herb which is extensively grown in India. It is a self grown erect or spreading herb found all over the country. This plant is a small bush which grows up to 1.5 meters tall and has bi-pinnate leaves with around 7-15 leaflets (Orwa et al.,2009) . It

can be used as a major ingredient in herbal preparations. Whole plant and many parts of *Tephrosia purpurea* are used in Siddha and Ayurvedic medicines to treat many diseases like bronchitis, pimples, bilious febrile attack, diarrhea, jaundice, gonorrhea, kidney disorders and disease of blood, heart and spleen (chaudhri et al., 2012).

According to pharmacological studies, *Tephrosia purpurea* has antioxidant (Kumar et al., 2011), antimicrobial properties (Rangama et al., 2009). Ayurvedic literatures described *Tephrosia purpurea* as 'Sarwa wranvishapaka' that means it has characteristic of healing all types of wounds (Lodhi et al., 2006). In this genus, the plants are distributed in arid, tropical and sub-tropical areas of the world. This plant is astringent, bitter, thermogenic, acrid and antipyretic.

Moreover, *Tephrosia purpurea* is a major component of some formulations like Yakrifit and Tephroli which are used for liver diseases. Traditionally, it is used to treat many types of wounds and gastro-duodenal disorders (Deshpande et al., 2003). Its drug is consumed in the stiffness of chest and cough.

Different parts of the plants have different medicinal uses like its root's decoction is helpful in obstruction and enlargement of spleen, liver and kidney. In addition to this, it is useful in chronic diarrhea and dyspepsia (Hussain et al., 2013). Its gargle is useful in washing out the mouth. Also, the root is useful in various disorders such as skin related disorders, inflammation, flatulence, elephantitis, chronic fever, anemia, asthma, hemorrhoids, bronchitis, gingivitis, dysmenorrhea etc.(William et al.,2006).

T. purpurea roots and seeds possess medicinal effects like its infusion is utilized in anthelmentic oil. Significantly, it is used in the poisoning due to rat bite and skin disorders like leucoderma, itching and scabies (Pavana et al., 2009). Leaves are used in treating pectoral disorder, dyspepsia, syphills, piles and hemorrhoid (Singh et al., 2002). Besides, the whole plant is useful to cure ulcers, tumors, leprosy and different inflammatory and allergic conditions such as bronchitis and asthma (Upadhyay et al., 2010). Some of the times, this plant is prescribed as blood purifier for pimples and boils.

In India, *Tephrosia purpurea* is a very common and useful ingredient in the medicines of liver disorders. In addition to medicinal use, leaves of this plant are used as food silage in South Africa and India. Moreover in India, dry plant is used as fuel and its seeds are used as an alternative to coffee (Sharma et al., 2013). There are so many countries all over the world which use *Tephrosia purpurea* as green fertilizer for coconut, rice, vegetables and banana. It is used in Sri Lanka and India on large scale and on limited scale in Indonesia, Malaysia and southern China. While developed as green compost on saline-sodic soils in Rajasthan, it is able to lower the pH and reduce soil salinity (Patil et al., 2011). The scientific research and modern studies of *Tephrosia purpurea* is an important herbal treatment which has antimicrobial, antioxidant properties.

Rangama et al., (2009) presented their antimicrobial activity of *Tephrosia purpurea*. Primary testing of antimicrobial activity of this plant is performed against 3 standard cultures i.e. *Pseudomonas aeruginosa, Staphylococcus aureus, E. coli*. Moreover, one medical isolate of *Candida spp*. was executed with leaves extracts, roots and pods using the process of Disc Diffusion Bioassay'.This method isolates the antimicrobial property of ethanolic root and scientific isolates of Staphylococcus, two Pseudomonas strains and nine forms of coli were experimented by using 'Well Method'. In order to examine the minimum inhibitory concentration of extract, all the active extracts were tested using Minimum Inhibitory Concentration agar dilution mechanism. Additionally, the impact of plant development was supervised on the antimicrobial property of *Tephrosia purpurea*. In this study, it was found that Ethanolic root extracts were dynamic against two coli strains, *Pseudomonas aeruginosa* and two other Pseudomonas strains (Rangama et al., 2009).

Apart from this, a research was conducted on antioxidant activity *Tephrosia Purpurea* Ethanolic extract in two different cultures i.e. superoxide generation in-vivo and carbon tetrachloride-induced lipid peroxidation in-vivo. In addition to this, an ethyl acetate portion of the same was analyzed for anti-lipid peroxidation and radical scavenging activity. This experiment has shown that IC50 values in both cultures were decreased for ethyl acetate fraction as compared to ethanolic extract of this plant. Further, the examination was assisted by evaluating the in-vivo antioxidant activity for both its ethyl acetate fraction and ethanolic extract. From the study, it was concluded that ethanolic extract of *Tephrosia Purpurea* shows antioxidant activity in-vivo. Also, it can be stated that ethyl acetate fraction has enhanced antioxidant nature more than ethanol extract. One more investigation was conducted on ten traditional plants for their antioxidant activity. Its outcomes exhibited that the chemical form of plant is the major reason for both their hepatoprotective and free radical scavenging activity (Kumar et al., 2011).

Croton bonplandianum is an example of medicinal plants. It is from the family Euphorbiaceae and subfamily Crotonoideae. *C. bonplandianum* is a monoecious exotic weed. *C. bonplandianum*, commonly known as Ban Tulasi, jungle tulasi in Bengali, kalabhangre in Hindi (Srivastava et al., 2010). This plant is about 60 cm high perennial herbs and can be found in waste lands and road side areas. It is a native plant of Paraguay, Southern-America, Pakistan and India. In India this plant is often called Ban Tulsi locally in sub Himalayan region of West Bengal. Generally *C. bonplandianum* distributed in the wasteland of tropical and subtropical regions of Madhya Pradesh (Qureshi et al., 2012). These plants produce flowers and fruits between Septembers to December (Reddy, 1995).

India has repellent properties against the insects and also possesses medicinal values in different regions (Nishanta et al., 2002). Its antiseptic properties are considered in the treatment of wounds and to stop bleeding of cuts by using its stem latex (Das et al., 2008). It also has antimicrobial

activities making its uses in skin disease treatments (Chandel et al., 1996). Myristoyl phorbol acetate (MPA) and 12 - Orthotrideconeoly-phorbol-13-acetate (TPA), diterpines, phorbolester are found in the seed of *C Bonplandianum*. TPA has carcinogenic properties which affects prostaglandin metabolism (Mandal et al., 2004).

Uses of croton species are diversified and used in the treatment of cancer, wound treatment, inflammation, hypertension, digestion issues, fever, ulcers, and weight loss (Salatino et al., 2007).

Traditionally *C bonplandianum* is also used for the treatment of various health issues: in hepatic problems (liver issues and jaundice), in respiratory problems (cold, cough, bronchitis, lungs infection, asthma), eye and skin diseases, rheumatoid, gastro intestinal disorders (acute constipation, cholera, boils, bowel, diarrhea, dysentery, insanity, abdominal dropsy, internal abscesses), scurvy, malaria, chicken pox, analgesic (pain management) and many more diseases.

Its importance has been recognized from various literatures for cholagogue and purgative purpose. Extract from the leaves have been reported for the treatment of ulcer, cancer, venereal diseases and many more (Ramchandran et al., 2009).

Antioxidant Activity - Assay for nitric oxide scavenging activity NO was generated as a result of decomposition of sodium nitroprusside in an aqueous medium interacts with oxygen at physiological pH to produce nitrite ions, which are measured by using Griess reaction (Green et al., 1982). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with Naphthyl ethylenediamine was read at 546 nm and referred to the absorbance of the standard solution of potassium nitrite, treated same way with Griess reagent. The reaction mixture (3ml) containing sodium nitroprusside (10μM) in phosphate buffer and various concentrations of drug i.e., 10, 20, 40, 80, 100 μg/ml of ethanolic extract of *Croton bonplandianum* or ascorbic acid were incubated at 25 degrees C for 120 minutes. Control without test compound kept in an identical manner. After incubation 0.5 ml of the incubation solution was removed and diluted with 0.5 ml of the incubation solution was removed and diluted with 0.5 ml of Griess reagent (1% w/v sulphanilamide, 2% w/v H Po and 0.1% w/v Naphthyl 3.4 ethylene diamine dihydrochloride). The absorbance of the chromophore was read at 546 nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control of the test compounds (Jun et al., 2004).

The antibacterial screening of leaf, fruit, latex extracts and fresh latex of *C. bonplandianum* was carried out and 10% w/v test solution of leaf, fruit and latex of *C. bonplandianum* were prepared by dissolving 500 mg of each extract separately in 5 ml of sterile 10% Dimethyl Sulphoxide (DMSO). From this 25, 50, 75 and 100μl extracts contains 2.5, 5, 7.5 and 10 mg, respectively and fresh latex was taken for the analysis of antibacterial activity. The extracts of leaf, fruit and latex of *C.*

bonplandianum were loaded at different concentrations (2.5, 5, 7.5 and 10 mg) in the well on pre inoculated Mueller Hinton Agar (MHA) plates with respective bacterial cultures and incubated at 37ºC for 24 hours. Streptomycin (10 µg) was used as a positive control and the solvent 10% DMSO was used as negative control for this study. After incubation, the diameter of the zone of inhibition (mm) around the well was measured using zone reader (Vennila and Udayakumar, 2015). The antibacterial activity of the various solvent extracts of the leaf of *C. bonplandianum* against bacterial isolates showed best results at the concentration of 7.5 mg/75 µl and The aqueous leaf extract showed a maximum zone of inhibition 15±2 mm against S. aureus while the minimum zone of inhibition 10±1 mm against *P. aeruginosa* when compared to other bacterial isolates. The ethonolic leaf extract showed the highest zone of inhibition 22±2 mm against *E. aerogenes* and *E. coli* while the lowest zone of inhibition 16±2 mm was observed against *E. faecalis* when compared to other bacterial isolates. The acetone extract of leaf showed a maximum zone of inhibition 19±2 mm against *E. aerogenes* and *E. coli* while the minimum zone of inhibition 10±1 mm against *P. aeruginosa*. The chloroform extract of leaf showed 19±2 mm inhibition against *S. aureus* and *E. aerogenes* and the benzene extract of leaf showed 20±2 mm inhibition against *S. aureus* (Theodorides et al., 1976).

Amaranth (*Amaranthus spp.)* also known as 'Chaulai', is a sustainable food crop which has high agronomic advantages and high nutritional values has witnessed attention in recent years. It is a protector against chronic diseases and is well balanced food (Haddad et al., 2016)

Amaranthus, a tropical food plant is mainly found in Southern India and Himalayan foothills. Found in colors ranging from green, red, purple, gold, has an appearance like spinach (Patel and Gandhi, 2017).

The genus Amaranthus is from Amaranthaceae family and has approximately 70 species into grains, leafy and vegetables forms (Grundy et al., 2020). America counts for majority of Amaranth species while Asia, Europe, Africa and Australia count for only 15 species (Martinez et al., 2020). Due to its adaption and pest tolerant nature it can grow under different agro-climates like heat and drought (Calzetta et al.,2000) and is also found in tropical, subtropical and temperature climate zones (Rastogi and Shukla,2013).

Despite having high nutrient of grains and consisting lipids b/w 5-13%, fiber b/w 9 to 14% protein b/w 13-22%, mineral and vitamins (riboflavin, niacin and ascorbic acid), betalains (Montoya et al.,2015). Proteins found in amaranth has plenty amount of lysine, methionine which is related to typical cereals (Miguel, 2018) and natural source of squalene (polyunsaturated triterpene compound). It is recommended as ideal food crop for millions by WHO and agriculture organization of United Nations considering its well balance nutritional values and gluten free nature (Santiago et al., 2014).

Uses of amaranth are in multiple categories like flour (soup, gravies) (Singh and Singh, 2011), confectionary (pasta and bread) (Alvarez et al., 2010), flakes, candies as a cereal in food (Barca et al., 2010). To produce bear it can be malted or fermented and its leaves are used in meat, fish dishes and salad (Mlakar et al., 2010).

It was used as basic food ingredient in drinks making tortillas with maize combination and for therapeutic treatments in Mayan and Aztec empires in ancient times (Orona et al., 2019). Cosmetics, paper coatings, laundry starch and biodegradable films are some example of its uses in nonfood category (Singh and Singh, 2011).

In Vietnam it was used in salad preparations, petioles, soup and young tips for potherbs (boiled greens) (Hoang et al., 2020). While Ethiopians used amaranth to make thin porridge for babies and new mothers, kita and borde (unleavened bread and alcoholic drinks) (Emire and Arega, 2012). In United States it is used as a breakfast amaranth commodity (Rastogi and Shukla, 2013).

In India, its seeds are consumed with rice, laddoos and popped grains are used by mixing molasses and honey to create confectionary products (Kaur et al.,2010) and amaranth grain used as substitute to wheat considering its higher amount of fiber and protein (Dixit et al.,2011). In medicinal uses amaranth is used for ulcers, diarrhea, and swollen mouth and throat and to treat high cholesterol.

Antimicrobial Activity: the extracts from the ethanol and methanol were screened for antifungal activity by agar well diffusion method (Perez et al., 1990). The 72 hours old fungal cultures were grown on Potato glucose agar. 20 mL of Potato glucose agar was poured in every Petri dish. After solidification, 0.1 ml inoculum of the fungal strains (1-2 x 104 CFU/mL) was introduced on the surface of the agar plate and the wells were made by using sterile cork borer of size 6.0 mm. 0.1 mL of methanol and ethanol plant extracts were introduced in the wells. An incubation period of 3-7 days at 23°C was maintained for observation of antifungal activity of plant extracts. Methanol and ethanol were used as negative solvent control. The antimicrobial activity was evaluated by measuring the zones of inhibition of the microbial growth surrounding the plant extracts in the wells. The zones of inhibition were measured in millimeters. Antimicrobial activity was assumed in the presence of a growth inhibition zone $\geq$ 7mm (Mohammadi-Sichani et al., 2012). The tests were performed in triplicate to determine the reproducibility of the results. The complete antimicrobial analysis was carried out under strict aseptic conditions.

(Bulbul et al., 2011) evaluated the antioxidant potential of methanolic leaves of *A. spinosus*, which was further fractionated in the n-hexane, chloroform, and ethyl acetate fractions. They reported the DPPH scavenging ability of each fraction by estimating the concentration that will inhibit 50% of the free radical (IC_{50}). He reported the highest in the n-hexane fraction followed by the chloroform fraction with the ethyl acetate showing the least and thus the highest antioxidant activity. He

however established a significant antioxidant activity for each of the fractions. The study of Ashkor-kumar et al. (2010) established the ability of the leave extracts to scavenge DPPH, NO, ABTS, O^{2*}, and OH^{-}. In their investigation however, the methanol crude extract was used against DPPH radical, superoxide anion radical, hydroxyl radical and ABTS. The antioxidant activity was recorded as the concentration that inhibits 50% of the radical. DPPH (IC_{50} 87.70±3.53 µg/ml), O^{2*} (IC_{50} 98.80±1.40 µg/ml, Butylated hydroxyl anisole IC_{50} 1.40±0.17 µg/ml) OH^{-}(IC_{50} 106.25± 0.20 µg/ml, Mannitol IC_{50} 2950±0.20 µg/ml), NO (IC_{50} 88.70± 0.62 µg/ml), ABTS (IC_{50} 147.50± 2.61 µg/ml, Ascorbic acid IC_{50} 1.64±0.05 µg/ml). This observation showed a weak antioxidant activity of the extracts compared with the standard antioxidants used. Whereas Ashkor-kumar et al. (2010) reported weak antioxidant activity of *A. spinosus* leave extracts, it must be borne in mind that the extracts are arrays of components and isolation and purification of the phenolic constituent may increase the activity immensely.

OBJECTIVES OF STUDY

1. To collect the selected arid zone plants of Rajasthan.
2. To phytochemical screening of selected arid zone plants (Qualitative).
3. To attain bioactivity guided fraction of selected arid zone plants
4. To screen antimicrobial and antioxidant potentials of plant extracts.
5. To study the medicinal properties of selected arid zone plants.

METHODOLOGY

MATERIALS AND METHODS

Instruments will be used in this study like spectrophotometer (U.V., visible), water bath, electronic weighing balance, distill water assembly, dryer/ oven, vacuum desiccators, sieve, centrifuge, shaker, thermostat, pre-autoclaved antibiotic preparing discs, conical flasks, incubator, forceps, autoclave, and inoculating loop, Erlenmeyer flask, rotary orbital shaker.

Reagents

Hexane, Methanol, Ethanol, Chloroform, Glucose, Sodium phosphate buffer, Ascorbic acid, 1,1-Diphenyl-2-Picrylhydrazyl (DPPH), Sulphuric acid (H_2SO_4), Dimethyl Sulfoxide (DMSO), Hydrochloric acid (HCl), etc. will be used in this study.

Collection of plants

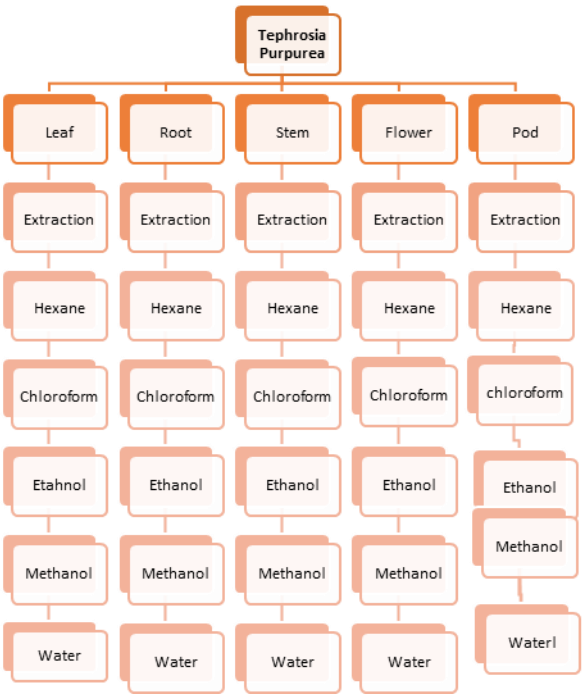
Different parts of plants (*T.purpurea, C.bonplandianum, Amaranthus spp.)* will be collected from various areas of Rajasthan.

Preparation of plant extracts

The samples of these plants (*T.purpurea, C.bonplandianum, Amaranthus spp.), w*ill be collected and washed with tap water first and then washed with distilled water. The sample leaves will be dried by air dryer. Then the dried plant leaves will be crushed and made in powder form by mechanical grinder. About 20 gms powdered part will be extracted in Soxhlet apparatus for 24 hrs with successive extracts than concentrated and dried (Harborne., 1998).

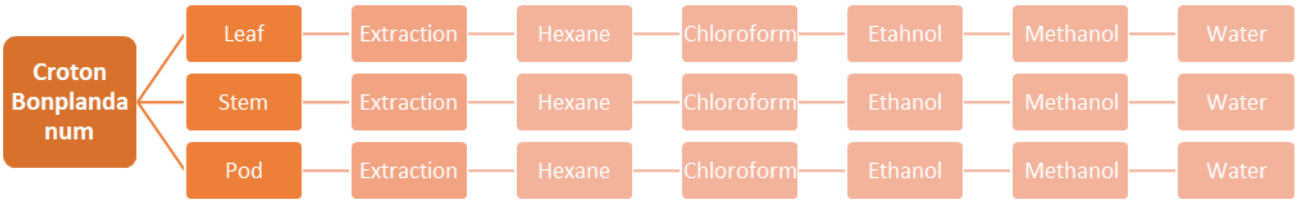
Test organisms

Gram-positive bacteria: *Staphylococcus aureus* and *Enterococcus faecalis* while *Salmonella typhi*, *Escherichia coli* , *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are Gram-negative bacteria. Clinical strain will be collected from the RUHS, College of medical sciences, Jaipur, Rajasthan. Solvent of ethyl acetate and n-hexane will be used as negative controls in the assays. Tetracycline/Streptomycin will include as antimicrobial agents in the study.



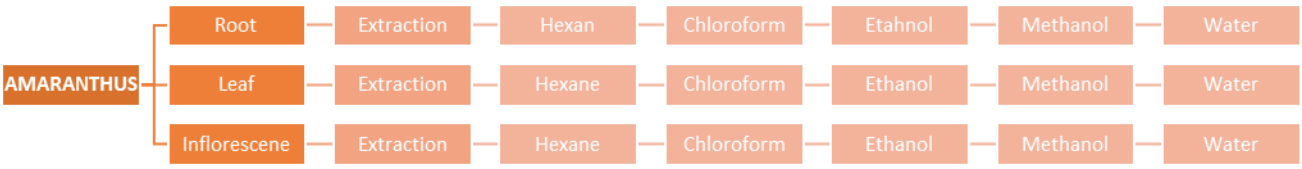
Source: Healthbenefitstimes.com

Figure 1:- *Tephrosia purpurea*



Source: Flowersofindia.net

Figure 2:- *Croton bonplandianum*



Source: Tropicalpermaculture.com

Figure 3:- *Amaranthus Spp.*

Phytochemical screening of plants leaves extract

Phytochemical screening of leaves extract for the presence or absence of phytochemical constituents like tannins, phenols, glycosides, alkaloids, steroids and flavonoids will be analyzed by standard method (Gupta et al., 1980).

Antimicrobial activity

The antimicrobial activity will be performed based on the phytochemical results. all extracts and reagents will be selected for antimicrobial activity and tested against Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative bacteria (*Salmonella typhi* , *Escherichia coli* , *Pseudomonas aeruginosa* and *Klebsiella pneumonia*) by agar disc diffusion method. The nutrient agar will be autoclaved for 15-20 minutes at 121˚C and 1 atm (Ncube et al.,2008) Will keep away the sterile nutrient media by maintaining temperature at 45-50˚C, after that 100 µl of bacterial suspension containing 108 colony-forming units (CFU)/mL will be mixed with sterile liquid nutrient agar and poured into the sterile petri dishes. Upon solidification of the media, filter discs (5 mm diameter) will be individually soaked with different concentration (as for example, 10, 25, 50, 75 and 100 mg/mL) of the extract and placed on the solidified nutrient agar media plates. The plates will be incubated for 24 hours at 37˚C. The diameter of the zone of inhibition (including disc diameter of 5 mm) will be measured with a scale. Each experiment will be performed three times to minimize the error and the mean values will be presented.

Antioxidant activity

The Diphenyl-picrylhydrazyl (DPPH) radical degradation method will be used to measure antioxidant activity (Sarker and oba, 2018). Briefly, will place 10 µl of leaf extract solution (in triplicate) in test tubes along with 1 ml of 250 µl DPPH solution and 4 ml of distilled water. Then tubes content will be mixed and allowed to stand for 30 min in the dark before reading the absorbance at 517 nm spectrophotometrically using an available instrument. And will follow the method described by Sarker and Oba for the $ABTS^+$ assay will include the stock solutions 7.4 mm $ABTS^+$ solution and 2.6 mm potassium per sulfate solution. After this process two stock solutions will mix in equal quantities to prepare working solution and allow them to react for 12 hour at room temperature in the dark condition. A 150 µl sample of leaf extract will be allowed to react with 2850 µl of $ABTS^+$ solution (1 ml $ABTS^+$ solution mixed with 60 ml methanol) for 2 hour in the dark condition. The absorbance will be taken at 734 nm spectrophotometrically against methanol using an available instrument. Antioxidant activity will be calculated as the percent of inhibition of DPPH and $ABTS^+$ relative to the control using the following equation:

Antioxidant activity (%) = (Absorbance of the control reaction - Absorbance of the test compound/Absorbance of the control reaction) *100

Will use Trolox as the reference standard, and the results will be expressed as mg trolox equivalent kg^{-1} dw.

EXPECTED OUTCOME OF THE STUDY

1. This study will help in determine the antimicrobial and antioxidant properties of different arid zone plants.
2. Antimicrobial properties of these plants species **(***T.purpurea, C.bonplandianum, Amaranthus spp.)*will help in skin disease treatments and its anticeptic properties will help in the treatment of wounds and to stop bleeding of cuts.
3. Study of these plants will help us to treatment of various health issues: in hepatic problems and in respiratory problems and treatment of ulcer, cancer, venereal disease.
4. This study will help us to find out presence or absence of phytochemical constituents (tannins, phenols, glycosides, alkaloids, steroids and flavonoids etc.) in plants extracts.

LIMITATIONS

To continue the study there are few limitations-

1. Plant possesses thousand of biomolecules but we will limit our study to few active extracts/ bioactives.
2. Amaranthus possess few species but we will restrict our study to red Amaranthus that is used as vegetable.

Year wise plan of work

1. Literature review 0 to 26 months
2. Survey of arid zone plants 0 to 2 months
3. Collection and selection of arid zone plants 2 – 8 months
4. Experimentation for phytochemical and antimicrobial studies 8 – 20 months
5. Statistical analysis 21 –23 months
6. Result and interpretation 24 – 26 months
7. Preparation of thesis 27 – 32 months

Year wise plan

Activities/Months	**0 – 2**	**2 – 8**	**8 – 20**	**21– 23**	**24 – 26**	**27 – 32**
Literature review						
Survey of arid zone plants						
Collection and selection of arid zone plants						
Experimentation for phytochemical and antimicrobial studies						
Statistical analysis						
Result and interpretation						
Preparation of thesis						

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